Evaluation of the dried pulp and peel composition of several citrus family fruits growing in Albania

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Abstract

The citrus family is one of the world's most important food crops. The fruit juice is widely used as a beverage and as a condiment. The fruit peels are used to produce citrus oil and as livestock feed. Because of the suitable climate, Albania is appropriate for the growing of the citrus family and these fruits are widely cultivated. The dried pulp and the peels of the citrus species are considered a second-hand material of the fruit. The fruit peels are a very interesting part of the fruit because of their high levels of some chemical compounds as vitamins. The most representative vitamins present in the citrus family fruits are the vitamin C and the vitamin A, but all the compounds of this category are present. Literature shows also that a large part of the alimentary compounds persists in the pulp after the juicing process. In Albania is it not commune to use the waste products of the citrus juices industry. In order to maximise the use of this portions, in this study was evaluated their chemical composition. Four different groups of citrus species grown in Albania were included in this study (*C. sinensis, C. limon, C. reticulata* and *C. paradisii*). The focus of our analysis were the moisture content (MC), the ash content (AC), the total polyphenolic content (TPC), the total flavonoid content (TFC), the total anthocyanin content (TAC), the vitamin A content (VAC), the vitamin C content (VCC) and the antioxidant activity (AA). Regarding the percentage of the above chemical compounds, the studied citrus species had differences not only between species but also between the fruit portions. The peel portion has higher levels of all compounds except for moisture. *Citrus paradisii* was the most representative specie, with the higher levels in the peels. On the other hand regarding the dried pulp, the richer specie was *citrus limon.* All the samples showed a high level of antioxidant activity in both peel and dried pulp water extracts.

1. Introduction

Citrus fruits are among the most popular fruits produced in tropical and subtropical areas of the world. Most citruses originated in China and Southeast Asia; lime probably developed in India. Members of this group consist of small trees or shrubs. This group contains orange, grapefruit, lime, lemon, and tangerine. The fruit is a berry made up of 10 to 13 segments called locules that are filled with juice sacs containing sugars and acids. If sugars prevail, the fruit may be very tasty, as are tangerines and most oranges. If acids prevail, the fruit may be quite tart, as are lemons and limes. Lemons and limes are often used as seasonings in cooking. Their juices may be diluted with water to which sugar has been added to make a refreshing drink. Citrus fruits are high in vitamin C.

Since citrus fruits are tropical or subtropical in origin, winter protection is a must. The lowest temperature at which growth in citrus occurs is 13 °C; the highest is about 38 °C. The best temperature range is 20 to 30 °C.

Hardiness differs according to species and sometimes variety. The tree can usually withstand temperatures 3 to 4 degrees cooler than the fruit. Ripe fruit can withstand lower temperatures than green or immature fruit. The duration of the cold period is as important as how low the temperature gets. It usually takes 3 to 4 hours at -2 to -3 °C to injure novel oranges. However, only 30 to 60 minutes at -3 °C will injure small lemons.

Citrus plants can be some of the most rewarding plants for the home gardener when you consider qualities of citrus. They produce deep emerald-green foliage, white, sweet-smelling flowers, and highly coloured fruit. Even citrus that is not edible can be grown as ornamental. Plant sizes range from small shrubs (such as the Meyer lemon) to large trees (such as the grapefruit). Provided you meet the climatic requirements (winter protection, light) and space requirements, you can grow any citrus variety.

Orange (*Citrus sinensis*) is an evergreen tree that grows 9-10 m tall (15). The leafs have oval shape and are 4-10 cm (6). The fruit may have different form and size but it is made up of 10 segments each one containing 6 seeds (10). Orange is a good source of vitamin C, but it contains also: carrotenoids, flavonoids (1) and a small number of volatile compounds like aldehydes, ketones, esters, terpenes and alchohols (9). The fruit pH range varies from 2.9 to 4 (4, 13)

Grapefruit (*Citrus paradisii*) is hybrid subtropical specie originated in Barbados (3). The fruit is medium-sized and has few, if any, seed. It is light yellow with a red blush at maturity. The fruit holds well on the tree. Harvest time ranges from December through May. It may be grafted onto the tri - foliate orange under stock. Grapefruit is a good source of vitamin C (7, 14), pectic fibres (5**)** and antioxidants (8, 14). Recent studies have shown a high activity on the reducing of the blood cholesterol levels (11,14).

Lemon (*citrus limon*) is an evergreen tree with yellow egg-shaped fruits. The fruit can be used as juice or in culinary and baked products. Lemon juice contains 5-6% citric acid but also vitamin C, polyphenols, terpenes and tannins (12)

Citrus reticulata (*Citrus reticulata*) is an evergreen tree with pole flat small fruits that are less tolerant to the climatic condition than the tree. The skin is of a deeper orange colour than regular oranges and is easily removed from the fruit. Citrus reticulata is used in food technology for the production of ice-cream, gelatine and gums.

Because of the high content of usefull compounds found in citrus fruits the aim of this study has been the determination of the percentage of several chemical compounds as polyphenols, anthocyanins, flavonoids and vitamins. As result it has been found that grapefruits peels are reacher than the other samples in the analysed compounds. On the other hand, the samples from lemon dried pulp resulted with higher ammounts of the analysed compounds.

2. Materials and methods

2.1 Sample preparation

The aim of this study has been the analysis of the chemical composition of several citrus family fruits grown in Albania. The focus of this study was on four species of citrus family as: orange (*Citrus sinensis*), lemon (*Citrus limon*), mandarine orange (*Citrus reticulata*) and grapefruit (*Citrus paradisii*).

About 1 kg sample of each fruit has been purchased in the local market, with all fruits having the same dimensions. After being weighted the fruits have been peeled and squeezed out and then placed on thermostat (WTB binder) in 80°C for 24 hours.

2.1.1 Peel an dried pulp treatment

After temperature treatment the peels and the dried pulps were blended with a Moulinex Masterchef 750 apparatus.

2.1.2 Water extracts preparation

Powder samples were weighted and an amount of 10g was extracted with 150ml distilled water (Heidolph promax) two times of 60 minutes each. After extraction the samples were centrifuged 1500g/min for a period of 10 minutes (Centrifuge universal 16A) and then filtered in a vacuum filtering apparatus.

2.1.3 Organic solvent extracts preparation

Powder samples were weighted and an amount of 10g was extracted with petroleum benzene (50-70°C) in a soxhlet apparatus.

2.2 Moisture determination

Moisture determination was measured as the difference in the weights before and after temperature treatment in 80°C for 24 hours.

2.3 Ash determination

The total mineral content method is based on the burning of the samples in a furnace in 600°C overnight. For the determination of ash content, a 5g of sample was used and the content was calculated as the difference between the weights before and after temperature treatment.

2.4 Total phenolic content (TPC) determination

Total phenolic content (TPC) was calculated according to the Folin-Ciocalteau method (**75**). An amount of 150 µl and 300 µl was added with 1ml ethanol and 5 ml distilled water. After vortexed to the the solution was added 0.5 ml Folin-Cioealteu reagent and vortexed again. After 3 minutes was added 1 ml sodium carbonate (Na2CO3 5g/l). The solution was kept in a dark place for 60 minutes and then the absorbance was measured in a photospectrometer at 725 nm length wave. As blank was used distilled water and the results are expressed as gallic acid equivalet.

2.5 Total anthocyanin content determination. Percaktimi i sasise se antocianeve totale

The total anthocyanin content was determinate using the method proposed by Di Stefano (2). The samples was diluted (250 µl /5 ml, 500 µl /5ml, 1000 µl /5 ml) in a solution containing ethanol, distilled water and concentrated hydrochloridic acid (70/30/1 v/v/v) and then was measured the absorbance at a length wave 540 nm. The dilution solution was used as blank and the results are expressed as malvidine-3-glucoside equivalents using the formula proposed by Di Stefano:

TA540 nm (mg/ml) = A540 nm 16.7d

TA540 nm(mg/ml) is the total anthocyanin content in 1 ml extract
A540 nm is the absorbance value measured at 540 nm length wave
d is the diluiton rate

2.6 Total flavonoid content determination.

For the determination of the total flavonoid content has been used a colorimetric method based on aluminium chloride ($AlCl_3$). An amount of 1 ml extract was placed in a laboratory flask and then added 4 ml distilled water and 0.3 ml of aluminium sodium nitrate ($NaNO_2$ 0.5 g/l). After 5 minutes was added 0.3 ml of aluminium chloride solution ($AlCl_3$ 1g/l). After 6 minutes was added 2 ml of a sodium hydroxide solution (NaOH 1 mol/l) and the volume was brought at 10 ml with distilled water and vortexed. Distilled water was used as blank and the absorbance was measured at 510 nm length wave. The results are expressed as catechine equivalents mg/g CE.

2.7 Total vitamin A determination.

10 ml of each sample was centrifuged, added with 10 ml KOH and vortexed for 1 minute. Then the flasks was placed on water bath in 60°C for 20 minutes and cooled. After cooling was added 10 ml xylene and vortexed again for 1 minute. Samples were centrifugated (1500rpm for 10 minutes) and the absorbance A1 of the supernatant was measured at 335 nm length wave using xylene as blank. Then the samples were placed under UV radiation for 30 minutes and measured the absorbance A2 at 335 nm length wave using xylene as blank. The vitamin A concentration was determined using the equation:

Cx = (A1-A2) x 22.23

22.23 is a constant value determined on the absorbance coefficient of a xylene 1% vitamin A solution measured at 335 nm length wave.

2.8 Total vitamin C determination.

A rapid and practical method for the total vitamin C content determination is the iodine titration method. Samples water extracts are centrifugated and filtrated and then 5 ml are added with 20 ml distilled water and 2 ml of a water solution containing 1% starch. The mixture is titrated with solution 0.01N iodine. 1 ml mixture is equivalent of 0.88 mg ascorbic acid.

2.9 Antioxidant activity determination.

All samples were tested for the antioxidant activity using the DPPH (2,2-difenil-1-pikrilhidrazil) method. An amount of 50µ and 100µl of each water extract was placed in a laboratory flask and added with 3 ml DPPH methanol solution (0.04g/l). After measuring the absorbance A1 at 517 nm length wave the samples were placed in dark for 60 minutes, after 60 minutes was measured absorbance A2 at 517 nm with methanol used as blank. The results are calculated using the formula:

AA = (ABScontrol – ABSsample/ ABScontrol) x 100

3 Results and discussion

3.1 Moisture content.

The aim of this study was the analysing of the characteristics of some citrus family fruits growing in Albania. The results (Table 3.1) show that the moisture content of all samples is not affected by significant changes. The peel moisture content varies within a range 70-80% with mandarin Citrus sinensis samples showing the lower content (68.65%) and the Citrus paradisii samples showing the higher levels (79.89%). Regarding the pulp samples the trend is the same as with Citrus reticulata samples showing the lower content (84.58%) and the Citrus paradisii samples showing the higher levels (94.98%). The results of this study agree with the literature where pulp moisture content of citrus family fruits is higher than peel level.

Sample	**Moi.C (Pe)**	**Moi.C (Pu)**
C. sinensis	74.04**%**	92.9**%**
C. limon	77.86**%**	93.5**%**
C. reticulata	68.65**%**	84.58**%**
C. paradisii	79.89**%**	94.98**%**

Table 3.1 Moisture content in citrus family fruit pulp and peel

3.2 Mineral content.

Object of this study has been the mineral content of the samples. The results show a high level of ash in Citrus paradisii compared with the other fruits either in pulp (2%) or peel samples (4%). On the other hand the lower levels of ash have been found on the Citrus sinensi samples. Citrus reticulata and Citrus limon samples show comparable levels of mineral content. Regarding the mineral content peel samples are richer than pulp samples. The results are expressed both in g/100 g and in percentage and are listed in table 3.2.

Sample	**Min.C (Pe)**	**Min.C (Pe)**	**Min.C (Pu)**	**Min.C (Pu)**
C. sinensis	0.08g	1.6%	0.06	1.2%
C. limon	0.12g	2.4%	0.07	1.4%
C. reticulata	0.1g	2%	0.05	1%
C. paradisii	0.2g	4%	0.1	2%

Table 3.2 Mineral content in citrus family fruit pulp and peel

3.3 **Total phenolic content (TPC).**

The results regarding the phenolic content of the citrus family fruits pulp and peel show a high amount of these compounds in all samples. The trend of the phenolic content is not the same in pulp and peel samples. In the case of peel samples the fruit with higher levels appears to be Citrus paradisii (1.764mg/ml) and the one with the lower levels is Citrus reticulata (0.879mg/ml).

On the other hand in pulp samples the fruit with higher levels of total phenolic content is Citrus limon (1.896mg/ml) with Citrus paradisii showing the lower levels (0.877mg/ml). Citrus sinensi and Citrus paradisii show higher levels of phenolic compounds in peel samples when in the Citrus limon and Citrus reticulata the opposite is true. When we analysed the total phenolic amount (pulp and peel) Citrus limon fruit resulted with higher levels and Citrus reticulata shows low phenolic content compared with the other fruits. Comparing pulp and peel samples the results are divergent. Citrus sinensi and Citrus paradisii peel samples resulted with higher amounts of phenolic compounds than pulp samples while in Citrus limon and Citrus reticulata pulp is richer than peel in polyphenols. Table 3.3 shows the results of the phenolic content in the peels and pulps of the citrus family fruits analysed.

Sample	TPC(Pe)	TPC(Pu)
C. sinensis	1.085 mg/ml	0.962 mg/ml
C. limon	1.386 mg/ml	1.896 mg/ml
C. reticulata	0.879 mg/ml	1.105 mg/ml
C. paradisii	1.764 mg/ml	0.877 mg/ml

Table 3.3 Phenolic content in citrus family fruit pulp and peel

3.4 Total anthocyanine content (TAC).

The total anthocyanine content has been determined using yhe method proposed by Di Stefano. The data of this study show that in the citrus family fruits the percentage of anthocyanine compounds is low (Table 3.4). The trend of the anthocyanine content is not the same in pulp and peel samples. In the case of peel samples the fruit with higher levels results Citrus paradisii (0.198mg/ml) and the one with the lower levels is Citrus reticulata (0.067mg/ml).

On the other hand in pulp samples the fruit with higher levels is Citrus limon (0.237mg/ml) with Citrus paradisii showing the lower levels (0.059mg/ml). Citrus sinensi and Citrus paradisii show higher levels of anthocyanine compounds in peel samples when in the Citrus limon and Citrus reticulata the trend is the opposite. When we analysed the total anthocyanine amount (pulp and peel) Citrus limon fruit resulted with higher levels and Citrus reticulata shows low anthocyanine content compared with the other fruits. Comparing pulp and peel samples the results are divergent. Citrus sinensi and Citrus paradisii peel samples results with higher amounts of anthocyanine compounds than pulp samples while in Citrus limon and Citrus reticulata pulp is richer than peel in anthocyanine. Table 3.3 shows the results of the anthocyanine content in the peels and pulps of the citrus family fruits analysed.

Sample	TAC (Pe)	TPC (Pu)
C. sinensis	0.156 mg/ml	0.069 mg/ml
C. limon	0.138 mg/ml	0.237 mg/ml
C. reticulata	0.067 mg/ml	0.089 mg/ml
C. paradisii	0.198 mg/ml	0.059 mg/ml

Table 3.4 Anthocyanine content in citrus family fruit pulp and peel

3.5 Total flavoniod content (TFC).

Object of this study has been the comparative evaluation of the total flavonoid content of the citrus family fruits analysed. Absorbance results (Table 3.5) show that Citrus limon is the fruit with the higher amounts of these compounds both in peel and pulp samples. The fruit with lower concentration of these compounds seems to be Citrus sinensi. Citrus sinensi and Citrus paradisii peel samples contain lower amounts of flavonoids than pulp samples while Citrus limon and Citrus reticulata seems to have same percentage of these compounds in peel and pulp samples. The results listed in table 3.5 are expressed as absorbance value.

Sample	Abs (Pe)	Abs(Pu)
C. sinensis	0.226	0.118
C. limon	0.420	0.455
C. reticulata	0.104	0.123
C. paradisii	0.326	0.120

Table 3.5 Absorbance value for the flavonoid content in citrus family fruit pulp and peel

3.6 Vitamin A content.

For the determination of the vitamin A content the method used is the one proposed by Rutkowski. The data of this study show that peel samples contain large amounts of vitamin A compared with pulp samples (table 3.6). The Citrus reticulata (5.49mg/ml) and Citrus limon (5.06mg/ml) peel samples manifested higher levels while Citrus paradisii samples resulted with the lower levels (2.66mg/ml) of vitamin A. In the case of pulp samples the trend is similar as with the peel samples. Citrus reticulata results to be with higher levels (1.63mg/ml) while Citrus paradisii shows lower concentration (0.46mg/ml). The data for the vitamin A content are listed in table 3.6.

Sample	**Vit. A (Pe)**	**Vit. A (Pu)**
C. sinensis	3.55 mg/ml	0.57 mg/ml
C. limon	5.06 mg/ml	1.50 mg/ml
C. reticulata	5.49 mg/ml	1.63 mg/ml
C. paradisii	2.66 mg/ml	0.46 mg/ml

Table 3.6 Vitamin A content in citrus family fruit pulp and peel

3.7 Vitamin C content.

The present study has analysed another important characteristic of these fruits such as the vitamin C content. The determination of the percentage of this compound in the samples has been carried out using the iodine titration method. The results (table 3.7) show a higher concentration of vitamin C in the peel samples than the pulp ones. The Citrus paradisii peels show the higher content of vitamin C (0.42 mg/ml) while the Citrus limon peel samples resulted with the lower levels (0.30 mg/ml). In the case of the pulp samples resulted with slice differences with Citrus limon and Citrus paradisii resulting with the higher amount (0.28mg/ml) and Citrus sinensi with the lower levels (0.25mg/ml).

Sample	**Vit. C (Pe)**	**Vit. C (Pu)**
C. sinensis	0.37 mg/ml	0.25 mg/ml
C. limon	0.30 mg/ml	0.28 mg/ml
C. reticulata	0.33 mg/ml	0.26 mg/ml
C. paradisii	0.42 mg/ml	0.28 mg/ml

Table 3.7 Vitamin C content in citrus family fruit pulp and peel

3.8 Antioxidant activity (AA)

The antioxidant activity was one of the objects of this study. The determination of this parameter is based on the attitude of DPPH on scavenging the free radicals produced after oxidation. To determine the antioxidant activity were used two concentrations 50µl and 100µl but the data show that the raising of the concentration of the extract was inefficient. Also the antioxidant activity was measured in two different moments T0 and T60 after 60 minutes. The results (table 3.8) show that Citrus paradisii exhibited a higher antioxidant in T0 while the lowest was the Citrus sinensi antioxidant capacity either in peel and pulp samples. On the other hand Citrus limon expressed a higher antioxidant activity in peel samples while Citrus paradisii was the most efficient regarding the pulp samples. Despite the large differences the data show that pulp samples seem to have a higher antioxidant activity than peel samples.

Sample	**AA (Pe T0)**	**AA (Pe T60)**	**AA(Pu T0)**	**AA (Pu T60)**
C. sinensis	35%	65%	39%	66%
C. limon	56%	91%	46%	77%

C. reticulata	40%	64%	49%	77%
C. paradisii	59%	86%	69%	89%

Table 3.8 Antioxidant activity values expressed as the reduction of the absorbance in citrus family fruit pulp and peel

4 Conclusions

The focus of this study has been the analysing of several parameters of some citrus family species growing in Albania. All the samples were analysed for moisture content, mineral content, phenolic content, anthocyanine content, flavoniod content, vitamin A and vitamin C content and antioxidant activity. Finally it has been concluded that:

1. The data of this study show that Citrus paradisii is the fruit with the most suitable characteristics among the citrus family fruit analysed in both peel and pulp samples.
2. Among the citrus family fruits considered in this study Citrus reticulata peel samples were the poorer ones. In the case of the pulp samples Citrus sinensi exhibited the lower levels of all the compounds analysed compared with the other fruits.
3. All the analysed samples expressed high levels of polyphenolic compounds, vitamin A and C.
4. All samples showed a strong antioxidant activity with the Citrus paradisii samples showing the most powerful activity.
5. The comparative assessment of data highlighted that citrus family peel samples were richer in all the parameter analysed

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